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RESPONSE OF BLACKGRAM (*VIGNA MUNGO. L*) TO SEED BIO-FORTIFICATION AND FOLIAR NUTRITION INTERVENTION IN RELATION TO SEED QUALITY AND YIELD POTENTIAL

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ABSTRACT

Cowpea and horse gram, the underutilized legumes of the tropics with high nutritional quality and low cost were used for preparing the pulse sprout extract at Agricultural Research Station, Vaigai Dam, Theni during 2011-13. The biochemical properties of the pulse sprout extract was analyzed. Seed fortification was effected through soaking of blackgram in both horsegram and cowpea sprout 1, 2, 3 & 4 % extract @ 1:0.3 w/v (seed to pulse sprout extract) along with $ZnSO_4$ (100 ppm) and water for three hours followed by dehydration in a drying chamber at 30° C for three days to reach original moisture content (10 ± 0.25% on wet weight basis). The seed quality analysis revealed that blackgram seeds fortified with cowpea 2% extract enhanced the germination percentage over $ZnSO_4$ hardening and control. The best performed cowpea 2% and horsegram 3% fortification were forwarded to field trial and foliar spray with cowpea 1% and horse gram 2 % sprout extracts were imposed along with water and DAP 2% at two growth stages i.e., 35 and 50 days after sowing. The combined application of seed fortification with cowpea 2% + foliar spray with cowpea 1% increased the physiological parameters of plant dryweight, leaf area index, crop growth rate, relative growth rate and net assimilation rate. The same treatment was also reflected the similar trend of result for yield parameters *viz.,* days to 50 % flowering, number of pods per plant, seed yield and 100 seed weight over control. The experiment has proved that the seed fortification with cowpea 2% + foliar spray with cowpea 1% at 35 and 50 days after sowing increased the seed quality, physiological and yield parameters in blackgram.

KEYWORDS: Seed Fortification, Pulses Sprout Extract, Foliar Nutrition, Plant Growth, Yield, Seed quality

INTRODUCTION

Sprouting is the practice of soaking, draining and incubating the seeds until they germinate. Subsequent to imbibition of water, seed germination is triggered by enzyme activities which result in elaborate biochemical changes. Proteins break into amino acids, whereas carbohydrates are converted into simple sugars. Besides, water-soluble vitamins such as B complex and vitamin C are created[1]. Germinated grains are also rich source of enzymes *viz.*, α-amylase[2], phytase[3], other digestive enzymes[4&5], water soluble vitamins such as Thiamin, Niacin, Vitamin A, B complex, vitamin C, minerals[6] and soluble sugars[7]. Because of the favorable accumulation of such bioactive substances, seed sprouting has been identified as an inexpensive and effective technology for improving the nutritional potential of cereals and grain legumes.

Cowpea [*Vigna unguiculata*], is known as black-eye beans, are a good source of protein, energy and other nutrients in developing countries. Horse gram is an excellent source of iron and molybdenum[8] and both the pulses are rich

in protein and comparatively lower in cost. Pulse sprout extracts of horse gram and cowpea has been proved to bring about seed vigour when employed through seed fortification and to bring about increase in seed yield when sprayed on rice crop at optimum concentrations[9]. Hence a study was carried out to develop a technology by using the bioactive substances of horse gram and cow pea sprouts for seed treatment and foliar spray to achieve higher yield in blackgram.

MATERIALS AND METHODS

The experiment was conducted by adopting Factorial Randomized Block Design (FRBD) at Agricultural Research Station, Vaigai Dam, Theni during 2012-13. Commercially available horsegram and cowpea were utilized for preparing sprout extract.

Preparation of Pulse Sprouts Extract

The cowpea and horsegram seeds were soaked for 10hr and incubated in a wet cloth for overnight to enable sprouting and 100 g of sprouts were obtained from 50 g of dry seeds. Later, 100 g of sprouted seeds were ground in a mixer-grinder by using 100 ml of ice water refrigerated at 5^0C. The ground substance was squeezed through cloth bag and 100 ml extract of 100 % concentration was obtained and diluted in to required concentration for seed treatment and foliar spray.

Biochemical Analysis of Pulse Sprout Extracts

The pulse sprout extracts of 100 percent concentration obtained from both horse gram and cowpea were estimated for total antioxidants activity[10] soluble protein content[11], total soluble sugars[12], ascorbic acid (volumetric analysis) and minerals *viz.*, nitrogen, phosphorus, potassium, calcium, iron and zinc, by employing Atomic Absorption Spectrophotometer.

Seed Fortification Treatment

Blackgram seeds were soaked for three hours in both horsegram and cowpea sprout 1, 2, 3 & 4 % extract @ 1:0.3 w/v (seed to pulse sprout extract) along with $ZnSO_4$ (100 ppm) and water under aerated conditions at room temperature (26 $\pm 1^0$c). After soaking, the seeds were dried back to original moisture content. Immediately after treatment, germination test was carried out in paper medium in quadruplicate using 100 seeds for each treatment with four sub replicates of 25 seeds[13] in a germination room maintained at a temperature of 25 ± 1°C and RH 96 ± 2 percent with diffused light (approx. 10 h) during the day. Final count on normal seedlings was recorded on seventh day and percent germination computed. Rate of germination was calculated using the formula of Maguire[13].

$$\text{Rate of germination} = \frac{X_1}{Y_1} + \frac{X_2 - X_1}{Y_2} + \ldots + \frac{X_n - (X_n - 1)}{Y_n}$$

where X_n = Percentage germination on n^{th} count

Y_n = Number of days from sowing to n^{th} count

Observations were also made on shoot length (cm) (the distance between collar region to the tip of the primary leaf), root length (cm) (the distance between collar region to the tip of the primary root), vigour index[14]. Seedling dry weight was determined using normal seedlings for which seedlings were dried in a hot air oven maintained at 85°C for 48 h and cooling in a desiccators for 30 min and weighing in an electronic digital balance and mean of dry weight arrived and

expressed as mg 10 seedlings $^{-1}$.

Foliar Nutrition

Foliar spray experiment was conducted during 2012 at experimental fields of Agricultural Research station, Vaigaidam (10° 0' North and 77° 8' East and altitude of 242 M above MSL). The soil texture of the experimental field is deep ulfisol, pH and EC is 7.2 and 0.32 dSm^{-1}, respectively. Seed rate compensation was done to maintain the plant population per unit area[15]. The recommended crop management practices were followed. Foliar spray with horse gram and cowpea sprout extracts were imposed in three concentrations *viz.*, 1, 2 and 3 % along with water and DAP 2% at two growth stages i.e., 35 and 50 days after sowing.

Seed Fortification Cum Foliar Nutrition

The blackgram seeds were fortified with best performed treatment of cowpea 2% and horsegram 3% and forwarded to field trial along with $ZnSO_4$ and control. The best performed foliar spray concentrations of horsegram 2% and cowpea 1% during preliminary screening were given at 35 and 50 days after sowing along with DAP 2% and water spray. The recommended crop production technologies were followed

Dry weight of the plants was recorded at 40 days of sowing after drying in shade and oven at 80°C for 36 h. Leaf area index after 30 days of sowing and net assimilation rate between 30-50 days of sowing were determined. Yield parameters viz., days to 50% flowering and number of pods per plant were observed. Seed yield was recorded after drying to uniform moisture content (9 ± 0.25%). The data were subjected to an Analysis of Variance and treatment differences tested (test) for significance ($P \geq 0.05$)[16]. Wherever necessary, the percentage values were transformed to arc sine values.

RESULTS AND DISCUSSIONS

Biochemical analyses were conducted to find out the nutritive constituents of pulse sprout extracts of horsegram and cowpea. Estimations was made on soluble protein, total soluble sugars, ascorbic acid, total antioxidant activity, besides minerals such as nitrogen, phosphorous, potassium, iron, zinc and calcium, in both 100 per cent extracts of horse gram and cowpea (Table 1). The estimations revealed that between horsegram and cowpea, cowpea recorded higher total antioxidant activity (90 mg /100ml), total soluble sugars (1.05 %), soluble protein (3.0%) and ascorbic acid (1.9 g/100 g). Among the minerals also, cowpea recorded higher values for iron (10.00 g/100 ml), potassium (339 mg/ 100 ml), nitrogen (93.61 mg/100 ml) as well as phosphorous (520 mg /100 ml). However, horsegram sprout extract showed higher value for calcium (20 mg/100 ml) and zinc (91 mg/100 ml).

Germinated grains are also rich source of enzymes *viz.*, α-amylase, phytase, water soluble vitamins such as Thiamin, Niacin, Vitamin A, B complex, vitamin C , minerals and soluble sugars. The vitamin C content of wheat, soybean and alfalfa increased 54%, 218% and 919%, respectively, after germination for 96 h at 28 °C in the dark[17]. The content of phosphorous, potassium, zinc and copper increased significantly as result of germination in various legumes[18]. All the mineral elements, except manganese and copper were significantly affected by germination.

The germination was significantly influenced by the pulse sprout extract treatment. Blackgram seeds fortified with cowpea 2% extract enhanced the germination by 5, 7 and 13 % over $ZnSO_4$ hardening, water soaking and control, respectively. Horse gram 3% extract also recorded the maximum value for germination (93 %) than the water soaking (88 %) and control (82 %). Speed of germination was also hastened by the same concentration of cowpea (13.7) and horsegram

extract (13.5). Seed fortification with different concentrations of cowpea extract recorded higher values for vigour and viability parameters when compared with water soaking and the effect was much better than control (Figure 1)

The experimental results revealed that seed fortification with pulse sprout extract recorded better performance compared to control. This may be due to increased nutrients in pulse sprouts produced physiological effects on seed and thereby improve its emergence and productivity[19] . In this experiment it was found that the seed invigouration with cowpea 2 % extract recorded higher values for all the seed quality parameters. This might be because of bioactive substances present in the extracts are more optimum for germination enhancement and the content of phosphorous, potassium, zinc and copper increased significantly as result of germination in various legumes. He also observed that germination of a wide array of legumes significantly improved the thiamine content of sprouted legumes. Nitrogen containing compound might have stimulated the germination by increasing the seed cytokinin content occurring naturally in seeds, which interacted with growth inhibitors and enhance the metabolic process, leading to higher germination[20] .

During seed invigouration, the first phase of germination ends with completion of imbibition process and hence the time taken from sowing to emergence is much reduced[21]. The improvement in field emergence due to invigouration could also be ascribed to activation of cells, which results in the enhancement of mitochondrial activity leading to the formation of more high energy compounds and vital biomolecules, which are made available during the early phase of germination[22]. Thus it is obvious that the presence of bioacitve substances in sprouted cowpea and horse gram extracts such as, amino acid, vitamins and minerals could have enhanced the seed quality parameters in blackgram seeds as corroborated by earlier reports. The seed vigour extended due to fortification with pulse sprout extracts had resulted in better seedling growth as reflected in germination percentage, root length and shoot length.

Foliar spray experiment was conducted in blackgram with three concentrations *viz.*, 1, 2 & 3 % of cowpea and horsegram sprout extract. The efficacy of the pulse sprout extract, when assessed through foliar spray under field conditions, the treatments were very effective. Foliar spray with cowpea 1% extract recorded highest plant dry weight (19.25 g) followd by horsegram 2% concentration (16.24 g). The earliness of flowering was observed in cowpea 1% (33 days) and horsegram 3% treatment (34 days) while the water (36 days) and control (38 days) recoded late flowering (Table 2).

Number of pods per plant was significantly differed due to foliar spray treatments but control recorded the minimum number of pods per plant. Among the different treatments, the maximum number of pods were observed in the cowpea 1% (23.0), which was followed by horse gram 2% (20.0). Observations on 100 seed weight revealed that cowpea 1% extract recorded the maximum values (4.22g) than the water soaking (4.18 g) and control (4.16 g). The contribution of all the yield attributing factors had consummated in higher seed yield. Cowpea 1% spray recorded 28 per cent yield increase (1150 kg) over control (900 kg) (Table 2).

Based on the results obtained in the previous experiments best and next best levels in seed fortification and foliar spray of both cowpea extracts (1 and 2 %) and horse gram (3 and 4 %) were combindly adopted for performance assessement in both K*harif* 2012 and R*abi* 2012-2013. The result showed that irrespective of seasons, the pulse sprout extract treatments improved the physiological and yield parameters in blackgram. The pooled data showed that seed fortification with cowpea 2% + foliar spray with cowpea 1% at 35 and 50 days after sowing increased the physiological parameters of plant dryweight (20.32 g) (Figure 2), Leaf Area Index (4.89) (Figure 2), Crop Growth Rate (24.94 g $m^{-2}d^{-1}$) (Figure 3), Relative Growth Rate (59.94 mg g^{-1} d^{-1}) (Figure 3) and Net Assimilation Rate (0.64 mg cm^{-2} d^{-1}) (Table 3). The same treatment was also reflected the similar trend of result for yield parameters *viz.,* days to 50 % flowering (32 days),

number of pods per plant (45) and 100 seed weight (4.21 g) than control (36 days, 27 numbers & 4.15 g, respectively (Figure 3; Table 3 & 4). Seed fortification with cowpea 2% + foliar spray with cowpea 1% increased the yield up to 41 % (1215 kg) over control (860 kg) (Table 4).

Under field conditions, the fortified seeds of blackgram produced vigorous plants and foliar treatment with cowpea extract increased the physiological and yield components. In order to achieve the highest possible yield, the physiological parameters especially leaf area index must be higher to intercept more solar energy for higher dry matter accumulation[23]. Net assimilation rate as a measure of growth efficiency was highest in treated seeds than control and gain support from the work of Egli and Yu-Zhen–Wen[24]. It is the amount of biomass deployed into some storage organs like grain which is the ultimate objective of the best treatment. The reproductive output and drymatter accumulation are positively correlated[25] and in the present study the difference in drymatter accumulation was wider between treated and control. Though the effects of vigour on field establishment are many, information on the association between vigour and yield is meagre and assumes practical significance[26]. Reduced efficiency of sub cellular organelles such as mitochondria or chloroplast in low vigour seeds would explain the reasons for lesser yield in untreated seeds[27]. If seed vigour affects yield, its influence could be observed through some effect on growth parameters like dry matter production, photosynthetic efficiency and related factors. Higher values for physiological parameters in treated seeds could explain the efficient translocation of assimilates to the sink. The yield in control was markedly lower than that of pulsesprout extracts treatment. Therefore, the reason for the reduced yield in control can be assigned clearly due to low vigour seed.

Seed fortification with cowpea 2% extract for three hours followed by dehydration is suggested for maintaining maximum germination and seed quality parameters. The combination of seed invigouration with cowpea 2% + foliar spray with cowpea 1% at 30 and 45 days after sowing increased the seed quality, physiological and yield parameters in blackgram.

Table 1: Biochemical Analysis of Pulse Sprout Extract

Name of the Sample	Horse Gram	Cowpea
Total antioxidant activity (mg / 100 ml)	87.50	90.00
Total soluble sugars (%)	0.90	1.05
Soluble Protein (%)	1.59	3.0
Ascorbic acid (g/100g)	1.3	1.9
Calcium (mg / 100 ml)	20.00	14.00
Iron (mg / 100 ml)	8.25	10.00
Zinc (mg / 100 ml)	91.00	89.00
Potassium (mg / 100 ml)	291	339
Nitrogen(mg / 100 ml)	89.36	93.61
Phosphorus (mg / 100 ml)	410	520

Table 2: Influence of Foliar Spray Nutrition with Pulse Sprout Extract on Physiological and Yield Parameters in Black Gram ADT 3

Treatments/ Characters	Plant dry Weight (g plant^{-1})	Days to 50% Flowering	No of Pods/Plant	Seed Yield/Ha (kg)	100 Seed Weight (g)
Control	13.75	38	17	900	4.16
Water spray	14.20	36	17	920	4.18
DAP 2%	15.70	35	20	960	4.19
Cowpea 1%	19.25	33	23	1150	4.22
Cowpea 2%	17.53	34	20	1010	4.20
Cowpea 3%	14.86	36	19	980	4.21
Horsegram 1%	15.80	35	19	1000	4.21
Horsegram 2%	16.24	34	20	980	4.19

Horsegram 3%	13.66	37	19	970	4.19
Mean	**15.67**	**35**	**19**	**974**	**4.19**
SEd	**0.41**	**0.14**	**0.31**	**9.0**	**0.02**
CD (p=0.05)	**0.83**	**0.29**	**0.62**	**18.0**	**0.04**

Table 3: Influence of Seed Fortification Cum Foliar Spray Treatment with Pulse Sprout Extract on Physiological Parameters in Black Gram ADT 3

Treatments	Net Assimilation Rate (mg cm^{-2} d^{-1})					Days to 50% Flowering				
	Kharif 2012	*Rabi* 2012-13	Pooled Mean			*Kharif* 2012	*Rabi* 2012-13	Pooled Mean		
T1	0.54	0.46	0.49			36	36	35		
T2	0.50	0.42	0.46			37	35	36		
T3	0.59	0.53	0.57			35	34	35		
T4	0.57	0.50	0.53			35	34	34		
T5	0.68	0.62	0.64			33	33	33		
T6	0.66	0.61	0.63			33	34	34		
T7	0.62	0.55	0.58			34	34	34		
T8	0.60	0.53	0.55			34	35	34		
T9	0.55	0.50	0.51			36	36	36		
T10	0.42	0.39	0.40			37	38	38		
Mean	**0.57**	**0.51**	**0.54**			**35**	**36**	**35**		
			S	**T**	**SXT**			**S**	**T**	**SXT**
SEd	0.02	0.03	0.01	0.50	0.81	0.18	0.14	0.17	0.26	0.38
CD (p=0.05)	0.04	0.06	0.02	1.01	NS	0.37	0.29	0.35	0.56	0.79

Table 4: Influence of Seed Fortification Cum Foliar Spray Treatment with Pulse Sprout Extract on Physiological Parameters in Black Gram ADT 3

Stages	Seed Yield $^{-ha}$ (kg)					100 Seed Weight (g)				
	Kharif 2012	*Rabi* 2012-13	Pooled Mean			*Kharif* 2012	*Rabi* 2012-13	Pooled Mean		
T1	880	840	860			4.16	4.13	4.15		
T2	855	820	830			4.18	4.15	4.17		
T3	940	950	920			4.19	4.16	4.18		
T4	1060	1025	1040			4.20	4.17	4.19		
T5	1230	1200	1215			4.22	4.20	4.21		
T6	1120	1090	1100			4.20	4.19	4.20		
T7	970	950	960			4.19	4.18	4.19		
T8	955	925	945			4.18	4.15	4.17		
T9	880	820	860			4.18	4.16	4.17		
T10	725	710	720			4.13	4.11	4.12		
Mean	961	933	945			4.18	4.16	4.18		
			S	**T**	**SXT**			**S**	**T**	**SXT**
SEd	10.96	10.33	11.06	17.50	24.75	0.02	0.01	0.01	0.03	0.06
CD (p=0.05)	22.50	21.20	(23.09)	(36.51)	(51.63)	0.04	0.02	(NS)	(0.06)	(NS)

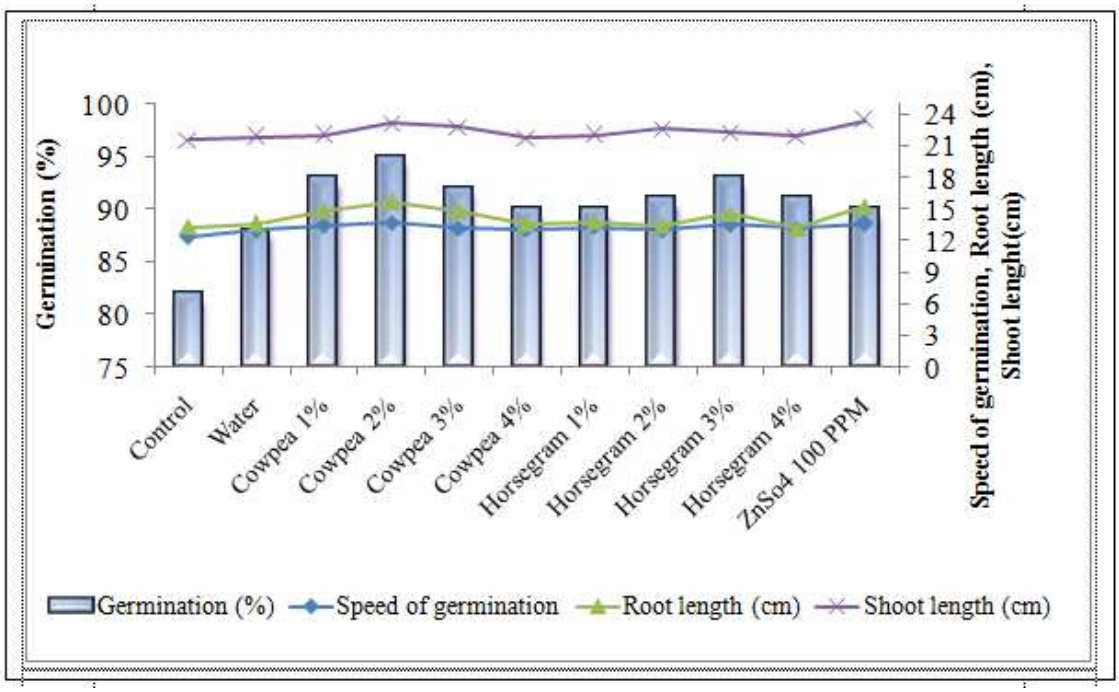

Figure 1: Influence of Seed Bio-Fortification Treatment on Seed Quality in Black Gram

Primary Axis: Germination (%),

Secondary Axis: Speed of germination, Root length (cm), Shoot length (cm)

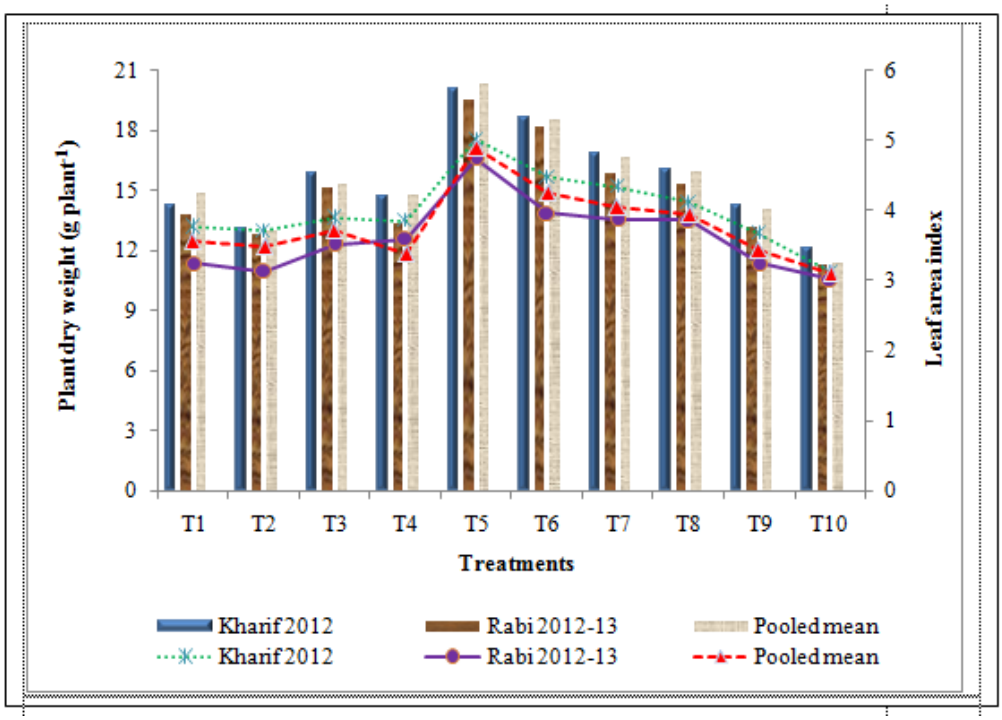

Figure 2: Influence of Seed Bio-Fortification Cum Foliar Nutrition on Plant Dry Weight and Leaf Area Index in Black Gram

Primary Axis: Plant dry weight (g plant^{-1}), Secondary axis : Leaf Area Index

Treatment Details : T1 – seed fortification with cowpea 2% ; T2 – seed fortification with horsegram 3% ; T3 –

foliar nutrition with cowpea 1% extract; T4 – foliar nutrition with horsegram 2% extract ; T5 – T1+ cowpea extract 1%, T6 - T1+ horsegram 2%; T7 – T2+ cowpea 1%; T8 - T2+ horsegram 2%; T9 – DAP 2%, T10 – Control

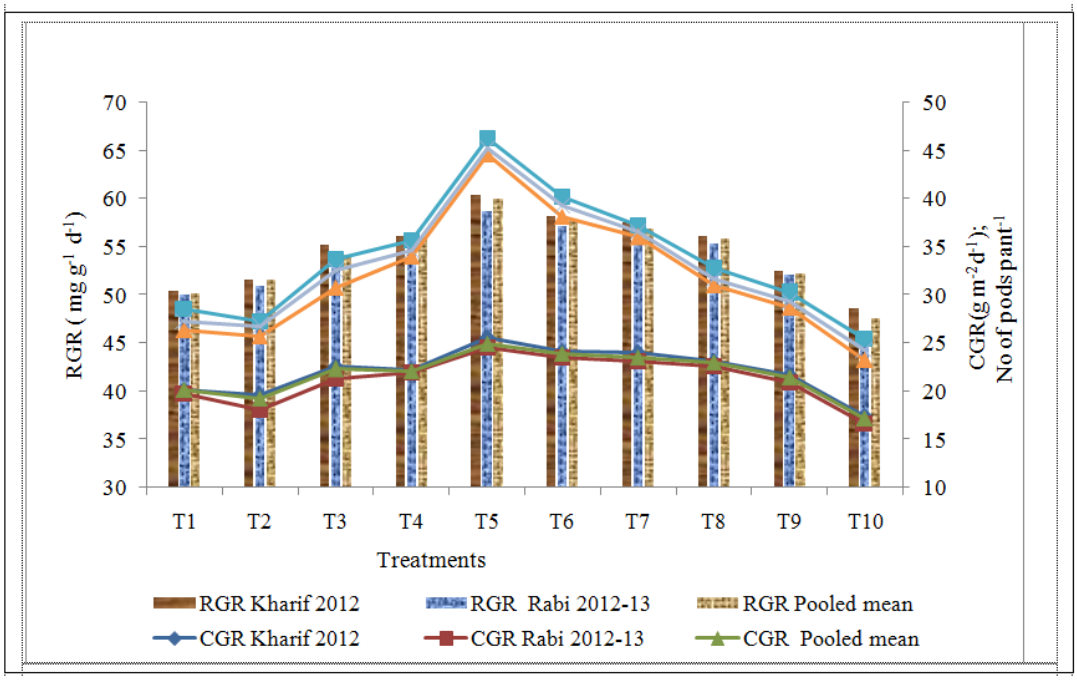

Figure 3: Influence of Seed Bio-Fortification Cum Foliar Nutrition on Yield Parameters in Black Gram

Primary Axis: RGR (mg g^{-1} d^{-1}), Secondary axis : CGR (g $m^{-2}d^{-1}$), No of pods $pant^{-1}$

Treatment Details: T1 – seed fortification with cowpea 2% ; T2 – seed fortification with horsegram 3% ; T3 – foliar nutrition with cowpea 1% extract; T4 – foliar nutrition with horsegram 2% extract ; T5 – T1+ cowpea extract 1%, T6 - T1+ horsegram 2%; T7 – T2+ cowpea 1%; T8 - T2+ horsegram 2%; T9 – DAP 2%, T10 – Control

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